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BY

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LII. ON THE PREPARATION OF COLLODION MEMBRANES OF DIFFERENTIAL PERMEABILITY.

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In the course of a study of the active principle of the extract of a parasitic fungus, the writer [Brown, 1915] attempted the fractional dialysis of the extract. For this purpose he first employed membranes prepared by impregnating filter papers with gelatin according to the method of Bechhold [1907]. By varying the strength of the gelatin employed a series of membranes of different permeability was obtained, none of which however was able to hold back completely the active principle of the fungus. Recourse was had, therefore, to collodion membranes. As this technique was new at the time to the writer, extremely discordant results were at first obtained. It soon appeared that with the same collodion solution very great differences in the properties of the resulting membranes could be produced by variations in the mode of treatment. A revision of this technique was undertaken, involving on the one hand a critical examination of the methods hitherto described and on the other the elaboration of methods which, to the present knowledge of the writer, constitute a new departure in the preparation of collodion dialysers.

It is not proposed to enter fully into the somewhat scattered literature relative to collodion membranes. A fairly complete account of the older literature on the subject is given in a paper by Bigelow and Gemberling [1907].

In practice collodion membranes are employed in the form either of flat sheets or of thimbles. Each type has certain special advantages. Thus the former lends itself more readily to osmometric work inasmuch as it can

easily be backed by some arrangement to take up the pressures developed; for purposes of dialysis the thimble form is simpler in manipulation as it does not require to be tied to any glass structure.

Collodion thimbles may be formed in two ways. A test tube is dipped into a collodion solution, and the resulting film left to undergo a certain amount of evaporation of the solvent. The film is then hardened by immersion in water, and the membrane so produced is stripped off by eversion. This is the method used by Malfitano [1904], by Duclaux [1907], etc. By other experimenters the membranes are formed inside the tube. The latter is filled with collodion solution which is then poured out, and the film of collodion remaining is allowed to drain for some time. The film is hardened by addition of water, and the resulting membrane readily shrinks away from the glass tube. This is the method described by Smith [1905], Bigelow and Gemberling [1907], Lillie [1907], Walpole [1915], etc.

Sheet collodion is prepared by pouring thin collodion solution over a glass surface which has been carefully levelled [Walpole, 1915] or on a mercury surface [Bigelow and Gemberling, 1907]. It is hardened in the usual way.

Numerous attempts have been made to prepare collodion membranes of uniformly varied permeability. These now call for detailed mention.

Bechhold [1907] prepared collodion films by impregnating filter papers with solutions of different strengths of collodion in glacial acetic acid. Impregnation was carried out *in vacuo*, and the filter papers after draining for a short time were hardened by immersion in water. The permeability of the membranes obtained was found to vary inversely with the strength of the collodion solution used.

Bigelow and Gemberling [1907] used a solution consisting of 3 g. commercial pyroxylin in 75 cc. ether + 25 cc. alcohol. They varied the permeability by varying the time allowed for evaporation of the solvents. No means of standardising this process were described.

Malfitano [1910] observed that the permeability of collodion thimbles diminished as they were allowed to dry in air; also that it could be diminished by heating the membrane in water. With similar treatment in other respects, a collodion richer in alcohol gave a more permeable membrane than one rich in ether. No details of a systematic nature were given.

Schoep [1911] describes a method which does not seem to have received the recognition that it deserves. He prepared membranes from pyroxylin-

castor oil solutions in alcohol-ether. The following represents the type of formula used by him:

Pyroxylin	4 g.	
Castor oil	3·5 g.	
Alcohol and ether	92·5 cc. (alcohol and ether in ratio 1 : 8).	

In order to vary the permeability of his membranes, Schoep added glycerol in various quantities to the pyroxylin-castor oil solution. The membranes were then formed inside a glass tube and allowed to drain and dry to constancy. On testing these membranes he found that their permeability increased with increasing glycerol concentration used.

Walpole [1915] relied mainly upon sheet collodion prepared by pouring a 3·5 times diluted stock solution of Schering's celloidin [13·45 per cent. by weight in equal parts of absolute alcohol and ether—thus approximately a 4 % solution] over a horizontal glass plate. The amount of solution used and the area of the resultant membrane being known, the amount of nitro-cotton per sq. cm. could be determined. This quantity (m) is used in describing the membrane. In order to obtain membranes of varied permeability Walpole varied the drying period. To determine how far drying had proceeded, he took sample portions of the film, washed them in water, and determined their thickness by means of a micrometer. With the same film, the thickness of the resulting membrane decreased as evaporation proceeded, so that by determining the former the progress of evaporation could be followed. Walpole found that the membranes so obtained could be described fully, for purposes of reference and reproduction and as a guide to their properties, by stating the kind of nitro-cotton used, the weight of it per sq. cm. they contained, and the "wetness," i.e. the ratio of the weight of a piece of film to its weight when dried to constancy.

The method of varying the permeability discovered by the writer is as follows. Collodion membranes are prepared and then completely air-dried whereby they become highly impermeable; they are then placed in solutions of alcohol in water for a suitable time. When subsequently washed in water these membranes are found to show a permeability which decreases regularly with the strength of the alcohol employed.

Thus while in the methods of manufacture hitherto employed, successful grading was a matter of skill which was not always definable, the present method is merely mechanical and the only skill required is in the making of the initial uniform air-dried membranes.

Standard method of preparation of air-dried membranes.

An 8 % solution of Schering's celloidin in an equal mixture (by volume) of absolute ethyl alcohol and ether is used. The membranes are formed on the outside of a test tube (1 cm. diam.). The latter is dipped in the collodion solution to a depth of 8–10 cm., and after a few seconds' draining is placed in a vertical position inside an inverted conical flask (½ litre capacity), being held by an ordinary cork which fits the neck of the flask. The purpose of the flask was in the first instance to limit the evaporation of the solvents so as to ensure an even film of collodion. By manipulation of the tube after insertion into the flask the length of the membrane can be increased. Drainage is allowed to proceed for 5 minutes after which the tube is withdrawn and *immediately* immersed in water. After a time (about 1 minute) the membrane is stripped off by eversion. For this purpose use is made of the "runnels" of collodion, which have by now become solidified. These on being cut across serve as handles in pulling the membrane off. The membranes are then trimmed, washed for a short time in water, and laid out to dry at laboratory temperature. Drying takes place with shrinkage and is completed overnight. The membranes are now ready for the alcohol treatment.

The shrinkage consequent on drying is a factor of very considerable importance in the present technique. A membrane which dries with excessive shrinkage will show at the finish a diminished surface over which diffusion may take place, along with increased thickness, and will not afford results comparable with those of a less shrunken membrane. Great care has therefore been exercised in examining the factors determining this shrinkage and in devising means for its proper control. What these factors are will appear from the following.

Characters of a normal membrane previous to drying off.

The characters which were examined in this connection were:

(1) permeability;

(2) water-content;

(3) thickness, before and after drying; shrinkage on drying, both longitudinal and circumferential.

A normal membrane is permeable to all crystalloids and allows slow diffusion of dextrin. It is more permeable at the closed end and may be relatively impermeable towards the open end. This is shown by the fact

that with certain dyes the inner surface of the membrane stains more rapidly in the neighbourhood of the closed end than towards the opening. Again the permeability of the outer surface is greater than that of the inner surface.

After washing in water, these membranes contain from 39·5 to 43·0 % of water (in terms of total weight).

It is obvious from the foregoing that with membranes of the present type the wetness gives no certain indication of the permeability as the membrane is not uniform on both sides.

The following method was adopted in measuring the shrinkage relationships of these membranes. The membrane was split from top to bottom along one side; the circumference was determined at top (open) and bottom (closed) ends, and also the thickness (by means of a micrometer) across the top, middle and bottom. In order to determine the degree of longitudinal shrinkage, a distance of 4 cm. was measured and marked by cuts in the long direction of the membrane. A repetition of these measurements in the case of the air-dried membrane gave data by which the shrinkage relationships could be followed.

The following series of measurements is typical of the present type of membrane (viz. a membrane which was drained 5 minutes in the flask and was immediately immersed in water). In the micrometer readings, each unit is equivalent to 5 μ.

Membrane before drying.

Circumference = 4·53 cm. at top; 4·50 at base.

Micrometer readings.

5·2, 5·8, 5·4, 5·5, 5·3, 5·0, 5·1 near top. Av. = 5·33.
5·7, 5·7, 5·6, 5·6, 5·8, 6·0, 5·9 near middle. Av. = 5·76.
6·3, 6·3, 6·6, 6·3, 6·2, 6·4, 6·2 near base. Av. = 6·33.

After drying.

Circumference = 4·15 cm. at top; 4·05 at base.
Marks 4 cm. apart have shrunk to distance 3·63 cm.

Micrometer readings.

4·0, 4·0, 4·0, 4·0, 4·0, 3·9, 3·7 across top. Av. = 3·95.
3·6, 3·7, 3·5, 3·6, 3·5, 3·5, 3·4 across middle. Av. = 3·55.
3·3, 3·3, 3·2, 3·2, 3·2, 3·2, 3·2 across base. Av. = 3·23.

In connection with this series of figures, the following points are noteworthy:

(1) At the same level of the thimble there is a variation in thickness, both in wet and in air-dried membranes. This variation is greatest near the open end. This can be ascribed to unevenness of drainage. It is better to discard the top part of each membrane.

(2) There is a variation in thickness from base to top of the same membrane.

In the case of air-dried membranes, thickness is found to increase from closed to open end. From a large number of observations, this rule has been found to be general. No such rule is however applicable to the corresponding undried membranes. Thus in the preceding case, the undried membrane was thicker towards the closed end, but nevertheless the closed end became the thinnest part of the membrane when the latter was dried off. Correlated with this we find the basal region to be the most permeable part of the wet membrane. In many cases however the undried membranes are found to show the same increase in thickness from the closed end upwards. The cause of these variations will be discussed below.

(3) The shrinkage relationships may be summarised thus:

Length before drying	=100	Length after drying	=90·75
Circumference before drying	at top =100 at base=100	Circumference after drying	at top =91·6 at base=90·0
Thickness before drying	at top =100 at base=100	Thickness after drying	at top =74·1 at base=51

Measurements of the above type have been carried out for a large number of membranes. The following general rules can be drawn:

(*a*) Longitudinal and circumferential shrinkage are approximately the same. Shrinkage is slightly greater at the closed end. Shrinkage in different membranes of the same type is remarkably uniform; thus of more than 30 measurements made, all lay within the limits 87 and 92, the majority lying between 88 and 90.

(*b*) Shrinkage in thickness is more variable. The thickness of the dried membrane varies from 50 to 75 % of the undried. Shrinkage in thickness is always greater than superficial shrinkage. This anisotropy in respect of shrinkage is invariable with these membranes.

The results so far obtained may be conveniently summarised at this stage.

By following the method described on p. 594, membranes can be obtained which on being air-dried present the following features: thickness = $20 \pm 5\,\mu$; tendency to greater thickness towards the open end; longitudinal and circumferential shrinkage very constantly 10 %.

Before drying, these membranes show a different permeability in their different parts; in this state therefore they are of no use whatever in cases where anything like exactness is required in dialysis work.

To attain a high degree of uniformity of the air-dried membranes certain precautions must be taken. The most important of these will appear from the following discussion of the effect of varying the conditions on the nature of the resulting membrane.

(i) *Effect of allowing free evaporation of the solvents.*

In the method of preparation described above, the membranes after draining in the flask are immediately plunged into water. The effect produced by even a short sojourn in the free atmosphere of the laboratory is very remarkable. This will appear from the following data:

The membrane is drained for 5 minutes in flask; on taking out it is held for 1 minute in air. Such a membrane will be described by the notation (5, 1); by the notation (5, 0) is meant a membrane which after 5 minutes' draining in the flask is immediately plunged into water.

Measurements of a (5, 1) *membrane.*

Circumference at top and bottom = 4·55 cm.
Micrometer readings 17·0, 17·0, 19·0, 18·7 across top. Av. = 17·9.
17·5, 16·3, 17·5, 18·9 across middle. Av. = 17·55.
15·3, 15·0, 15·9, 15·1 across base. Av. = 15·3.

After drying.

Circumference = 3·2 cm. at top; 3·1 at base.
Distance between 4 cm. marks = 2·8 cm.
Micrometer readings 8·0, 7·0, 6·5, 6·5 across top. Av. = 7·0.
7·0, 7·0, 6·3, 6·8 across middle. Av. = 6·8.
5·7, 6·1, 6·1, 6·1 across bottom. Av. = 6·0.

Thus the shrinkage relationships are summarised in the following table.

Length before drying		= 100	Length after drying		= 70·0
Circumference before drying	at top	= 100	Circumference after drying	at top	= 70·3
	at bot.	= 100		at bot.	= 68·1
Thickness before drying	at top	= 100	Thickness after drying	at top	= 39
	at bot.	= 100		at bot.	= 39

There is thus in this case excessive shrinkage on drying. Membranes of this type are before drying highly permeable—much more so than membranes of the type (5, 0). They are slowly permeable to aniline blue, strongly permeable to dextrin and allow crystalloids to pass with great rapidity. Correlated with this they show a high water-content; thus weight of dry membrane = 100; weight of wet membrane = 170.

The above figures are typical of all membranes of this type. The longitudinal and circumferential shrinkage borders very constantly on 30 %; the shrinkage in thickness approximates closely to 60 %.

A comparison of the measurements of a (5, 0) and a (5, 1) membrane is instructive. The unit of thickness is, as before, 5 μ.

(5, 0). Before drying. Thickness = 5–7.
After „ „ = 3–4.
Longitudinal and circumferential shrinkage = 10 %.
Shrinkage in thickness = 25–50 %.

(5, 1). Before drying. Thickness = 15–18.
After „ „ = 6–7.
Longitudinal and circumferential shrinkage = 30 %.
Shrinkage in thickness = 60 %.

It will be noticed that while shrinkage in thickness is greater in membranes of the second type, the final thickness of the latter when dried is about twice that of the former.

It will thus be seen that unequal evaporation of the solvents produces unequal shrinkage in the resultant membranes. The fuller discussion of these results will be postponed until the variation of a second factor has been examined.

(ii) *Effect of varying the time of drainage.*

The time of drainage within the flask was varied from 1 minute up to 10 minutes, and the resulting membranes tested as to their permeability, thickness in the wet condition, and shrinkage on drying. The following table gives the figures obtained. The notation in the case of the membranes is that already described (p. 597).

Membrane	Thickness when wet	Longitudinal and circumferential shrinkage	Thickness when dry
(1 0)	6–8	5–6 %	4·4–6·3
(1 ½)	17–19	20 %	5·7–6·9
(1 1)	19–20·4	25–27·5 %	6·7–8·0
(3 0)	6·3–7·9	7·5–10 %	3·7–4·8
(3 1)	17·0–20·8	28–30 %	6·2–7·2
(5 0)	5·3–7·1	8–12 %	3·3–4·5
(5 ¼)	14	22·5 %	5·1–5·7
(5 ½)	13·5–18	20–25 %	5·4–7·8
(5 1)	16·7–20·4	30–32 %	6·0–7·4
(5 2)	20·8	30 %	7·8
(10 0)	5·0–5·25	11–13 %	3·0–3·1
(10 1)	12·6–13·6	30 %	5·1–5·4

From the above table it is clear that by prolonging the period of drainage a slow increase of the shrinkage obtained on drying the resultant membrane takes place; that when once the membrane is taken out and allowed to

evaporate freely, a rapid change sets in, and the shrinkage soon reaches as much as 30 %. In this technique therefore it is imperative that the membranes be taken from the flask and immediately immersed in water. The rapid execution of this process involves a certain amount of dexterity only attainable by practice. The writer has convinced himself that it is quite possible to reduce the shrinkage variation to less than 5 %. Should it be considered necessary to bring this factor under better control, it is obvious that an arrangement could be set up whereby the films could be hardened without being exposed at any time to rapid evaporation of the solvents in the open air.

By lengthening the time of drainage the thickness of the resultant membrane diminishes, but only slowly. Even after 10 minutes' draining, the membranes when dried are thicker at the open end than near the closed end. Uniformity in thickness is a condition which is approximated to only after very prolonged drainage.

The membranes which have been allowed to evaporate freely for some time all show high permeability. In the case of membranes which are immediately transferred to water, permeability increases with time of drainage. Thus a (1, 0) membrane is impermeable, except at the closed end, to neutral red; a (10, 0) membrane shows a fairly uniform medium permeability throughout. With intermediate drainage, the membranes show a gradation from a more permeable region near the closed end to a less permeable region near the open end.

A membrane of the (1, 0) type when placed in water becomes strongly milky in appearance except just at the closed end which is clear. By longer drainage, the milkiness is reduced, and in the case of a (5, 0) membrane becomes very slight. Membranes of the strongly shrinking type are always glass-clear.

It is convenient at this point to explain the principles underlying the phenomena above recorded. For this purpose it is necessary to anticipate somewhat.

Membranes prepared from a solution of collodion in ether alone are highly impermeable, are white, have a low water-content, and show a very small shrinkage on drying. This statement applies all the more markedly when practically no ether is allowed to evaporate from the film before it is placed in water. Membranes made from a solution of collodion in alcohol alone are, on the other hand (unless a large proportion of the alcohol be allowed to evaporate), highly permeable, are clear, have a high water-content

and undergo strong shrinkage on drying. The various types of membrane obtained above represent intermediate stages of this series.

Now when a film of collodion on a test tube is exposed to free evaporation, the first result is the rapid diminution of the ether content. The membrane therefore lies near the alcohol end of the series, and a highly permeable, strongly shrinking membrane is obtained. Membranes of the type (1, 1), (3, 1), (5, $\frac{1}{2}$), (5, 1), (10, 1) are nearer to the alcohol end of the series, those of type (1, 0), (3, 0), (5, 0), (10, 0) are nearer to the ether end. That the (1, 0) membrane approximates more closely to an ether-collodion membrane than does the (10, 0) is due to the fact that the atmosphere inside the flask requires some time to reach equilibrium, and also to the fact that with the apparatus used a slow leakage of ether took place at the neck of the flask. That the basal (closed) end approximates more closely to the alcohol type of membrane than does the other is to be set down to the fact that in the earlier stages of drainage when evaporation is most rapid the closed end of the film (which is uppermost) has reached its limiting thinness, whereas the lower portion is protected by the excess collodion which is flowing past. This inequality, as has been already stated, is greatly diminished after about 5 minutes' drainage.

It will conduce to clearness at this stage if the essential features of the technique described above be emphasised. This is most conveniently done by stating the reasons which lead the writer to recommend each particular detail of the method described on p. 594.

It should be stated first of all that membranes of the strongly shrinking type are to be avoided for reasons of efficiency in dialysis. With equal dimensions at the start, the capacity of a membrane which has shrunk by 30 % bears a ratio $7^3 : 9^3 = 1 : 2{\cdot}1$ to that of one which has shrunk by 10 %—i.e. it is less than half. The thickness of the much shrunk membrane is also greater, so that with the same intrinsic permeability, diffusion is slowed down. It need hardly be added that in setting up any series of graded dialysers, membranes showing different degrees of shrinkage must be avoided.

Use of the conical flask. When a test tube is dipped into an 8 % collodion solution and withdrawn, a drop of collodion forms at the closed end of the tube. When the tube is placed vertically with closed end upwards, this drop must be made to travel the whole length of the film, otherwise extreme irregularity in thickness of the membranes results. When the films are allowed to evaporate freely there is a tendency for the excess collodion to stick half way, and very uneven drainage results. This tendency is

especially marked in warm weather. Again, even if drainage is fairly uniform, the membranes are found on drying to be of the strongly shrinking type. The use of the flask is to obtain even drainage along with a minimum of shrinkage on drying.

Strength of collodion used. With increase in concentration above 8 %, a tendency to uneven drainage sets in. Thus 10 % collodion after 5 minutes' drainage gives distinctly less uniform membranes than does an 8 % solution. With a 5 % solution, even drainage is soon secured. The membranes are however more liable to tear on stripping from the tube, and are found to shrink strongly. The latter feature is due to the fact that on account of the comparative fluidity of a 5 % solution, only a small amount of solution is carried into the flask on the tube, and consequently a considerable percentage of the solvent (especially ether) is evaporated before the atmosphere of the flask becomes saturated.

Time allowed for drainage. For the membranes normally employed, a period of 5 minutes' drainage was allowed.

For the first 1–2 minutes, drainage is still proceeding rapidly. From 3 minutes onwards only a very slow drainage takes place. Membranes which have drained 3 minutes show greater variation between closed and open ends than do membranes of the normal type. They also show a much greater tendency to milkiness. The latter objectionable feature is reduced to very small dimensions in the case of membranes which have drained 5 minutes. The only objection to drainage longer than 5 minutes is the time thereby taken up.

In carrying out the routine of preparing membranes, the experimenter cannot afford to abate any consideration which tends to uniformity. Each process in the manipulation should be standardised. In particular, the membranes should be made at a standard temperature (not higher than 20° C.), and as nearly as possible the same amount of solution should be taken up by the test tube in each case.

Summary. By carrying out the above routine, membranes can be obtained which show a shrinkage on drying of 10 ± 2 %. The dried membranes vary in thickness from 15 μ at the closed end to 20–25 μ at the open end; at any given level in the same membrane a variation in thickness of 10 % is liable to occur.

Treatment with alcohol.

The stock alcohol (ethyl) employed was of specific gravity 0·7956 (at 16° C.), corresponding thus to 99·36 % absolute (by weight).

By an x % alcohol in the following account is meant an alcohol-water mixture containing x cc. of stock spirit + $(100 - x)$ cc. water.

The air-dried membranes are soaked in the alcohol mixtures for 24 hours at 20° C.; they are then transferred to distilled water and thoroughly washed over a period of 1 day. They are then ready for use.

When uniform air-dried membranes are used, the permeability is determined by the concentration of alcohol employed. It may thus be fully specified in terms of the alcohol percentage. By the phrase "a 75 % membrane" is meant an air-dried membrane which has been soaked in 75 % alcohol for 24 hours at 20° C., and subsequently washed in water.

The series of membranes treated with increasing percentages of alcohol is thus a series showing gradually increasing permeability. The limits of the series are on the one hand 0 %, on the other about 97 %, above which point the membrane goes into solution. The most permeable membrane that has been systematically used is a 96 % one; 97 % membranes have also been prepared but are extremely fragile and apt to rupture. Membranes more permeable still have been prepared by allowing a shorter imbibition period in 98 % alcohol. It is possible therefore to extend the series, thereby obtaining extremely permeable membranes, but they are of doubtful value unless means be taken to support them and protect them from mechanical injury.

In the following work these membranes were set up in specimen tubes of 1·7–2 cm. diameter. In cases where estimations of diffusion were made, tubes of 1·8 cm. diameter were alone used. The membranes were filled with the solution to be tested to a distance of about 1 cm. from the top, and the space between the membrane and the tube filled to the same level with water.

The following substances were used:

NaCl(m), NH_4Cl(m), Na_2SO_4(m), $CuSO_4$ (sat.), $K_4FeC_6N_6$ (sat.), $K_2C_2O_4$(m), glucose (m and m/5), picric acid (sat.), $KMnO_4$ (0·5 %).

Bismarck brown, methylene blue, eosin, neutral red, safranine, aniline blue, Congo red, night blue (all 0·1 %).

Dextrin (0·5 %), starch (0·1 %), litmus (Kahlbaum).

All the solutions were made up in water.

In order to prove that a continuous gradation in permeability is obtained by the alcohol treatment, four main methods were employed.

I. Using a suitable crystalloid and membranes within a given range, the amount of endosmosis of water in a given time increases with the strength of alcohol used.

This method is capable of great elaboration, viz. by constituting the membrane an osmotic cell with capillary tube attached, and measuring the rate of endosmosis of water. This method of testing the variation of permeability with alcohol treatment, and the degree of uniformity of membranes treated in the same way, has not been carried out in detail as yet and only a few rough measurements of the alteration of level between the liquid inside the membrane and the water outside are at present available. In most cases no measurement was taken, but a note made to the effect that rate of endosmosis increased with strength of alcohol used. These measurements were made incidentally to determining the permeability of these membranes to various salts.

The following figures illustrate the type of result observed:

0 %, 60 %, and 70 % membranes set up with molar potassium oxalate.

After 24 hours, endosmosis of water in 0 % = 0·2 cm.
" " 60 % = 3·5 "
" " 70 % = 4·6 "

The increased endosmosis in 70 % takes place in spite of a slight passage of the oxalate through the membrane. In fact, increased endosmosis of water with strength of alcohol used takes place long after the membrane has become quite permeable to the osmotic substance. In such a case, of course, the endosmotic effect will prove to be transitory.

II. Within certain limits, as regards nature of membrane and nature of dye, the amount of staining of the membrane in a given time increases with the strength of alcohol used.

This method has proved of great value, especially for revealing what amount of irregularity in permeability may exist in different parts of the same membrane. The present test is certainly the most accurate from this point of view that is known to the writer. Though much use has been made of this test, only the cruder outlines are as yet worked out. The method lends itself readily to exact colorimetric work.

The following account of a particular experiment will illustrate how the method works out:

50 %, 60 %, 70 %, 80 % membranes set up with neutral red solution inside, water outside.

After 1 hour's staining, all membranes are thoroughly washed in water.

A continuous gradation in staining effect is seen, the latter being least in the 50 % and greatest in the 80 % membrane. The membranes are again set up with neutral red and left for 24 hours. After this time, the dye has passed through the 80 % membranes. On cutting all the membranes open, the difference in staining between the 70 and 80 % membranes is now found to be slight; a marked difference is still shown between 50 and 70 %. Even after prolonged staining, this difference is not effaced—that is, e.g. the intensity of staining of a 60 % membrane never reaches that of a 70 % one.

From an extended series of experiments of this sort, a continuous gradation in general staining effect with varying strength of alcohol has been established. This is readily done in the case of 0 %, 30 %, 40 %, 50 %, 60 % and 70 % membranes with neutral red solution; by 24 hours' staining with aniline blue, a similar gradation can be established in the case of 70, 75, 80, 85 and 90 % membranes. Staining of 80, 85, and 90 % membranes with neutral red takes place too rapidly to allow of a satisfactory differentiation.

In applying this method to the detection of irregularities in permeability of different parts of the same membrane, the latter should be stained with a dye which is unable to pass through it. Thus neutral red was employed in the case of 30–70 % membranes; aniline blue for 70–90 % membranes. In cases where the dye is able to pass through the membrane, the staining rapidly becomes so intense that variations are no longer noticeable.

The following are the results obtained:

(1) With 70 % and higher membranes, staining is completely uniform, apart from a limited number of small spots at which the membrane is less permeable.

(2) With membranes less than 70 % there is, in addition to the above irregularity, a tendency to uneven staining, the membrane being more permeable near the closed than near the open end.

(3) When the outside of the membrane is tested in this way, greater irregularity may occur. This irregularity is correlated with the presence of milkiness, the milky patches staining more strongly. This effect is shown equally well in the air-dried (0 %) membranes, and with such a dye as aniline blue. On cutting such membranes and examining microscopically, the staining effect is seen to stop in the middle of the membrane.

With glass-clear membranes the degree of staining of the outer surface is uniform and equal to that of the inner surface.

It may be remarked here that the presence of a number of small less permeable spots has been observed in the case of membranes of all kinds, no matter how made and no matter what the duration of the alcohol treatment. Their cause is unknown. From their small size, their effect on the general permeability of the membrane is negligible.

III. The amount of a particular substance passing through in a given time increases with strength of alcohol used.

Increased permeability with varying strength of alcohol has been followed over a range of 30 % to 96 % by measuring the rate of diffusion through the various membranes of certain substances. The membranes were set up as usual, the containing specimen tubes being chosen of equal diameter. The diffusing substance was placed inside, and the external space filled to the same level with water. The substances employed were such that no appreciable disturbance of levels (and volumes) was produced by endosmosis of water. Diffusion was carried on in a thermostat at 20° C.

(*a*) Diffusion of potassium permanganate (0·5 % solution). The amount diffused through in 24 hours was determined by titration with a 0·1 % $FeSO_4$ solution in dilute H_2SO_4.

Membranes—a double series of 30, 40, 50, 60, 70 %.

In all cases the tubes were filled to the same height, and at the end of the diffusion period the amount of solution inside the tube and outside the membrane approximated closely to 5 cc.

		Membrane				
		30 %	40 %	50 %	60 %	70 %
cc. of 0·1 % $FeSO_4$	Series *A*	0	1·6	10·6	31·2	76·5
	Series *B*	trace (< 0·10)	1·55	8·8	32·5	70·2

The difference between 60 % and 70 % is much greater than the figures represent. At the time of examining, equilibrium had nearly been reached between the outside and inside solutions.

The grading can also be seen by noticing how long a time elapses from filling the membranes till the first traces of colour appear in the liquid outside.

Thus in the case of the foregoing membranes, this happened within 1 minute in both cases with 70 %; in 4–5 min. in both with 60 %; in 25 min. in both with 50 %; in about 2 hours in both with 40 %; while in the case of 30 % membranes, nothing to a trace appears in 24 hours.

On washing these membranes staining is seen to have taken place; on

again air-drying them they are found to be impermeable to permanganate. The presence of holes is thereby excluded. It is probable that the membranes are slowly attacked—at least the permanganate becomes turbid when kept in a membrane for about a week.

(*b*) Over the range 70 to 95 %, the grading can be followed by using methylene blue. Over the range 70–80 % this has been done in considerable detail (see Uniformity tests below). Over the wider range grading is shown by the time required for the dye to appear in the outside liquid. The following figures illustrate this point:

70 %	75 %	80 %	85 %	90 %	95 %
about 6 days	1 day	8–12 hours	4 hours	70 min.	12 min.

(*c*) Over the range 85 to 94 %, with variations of 1 % in alcohol treatment a continuous gradation of permeability can be shown by using dextrin as diffusing substance.

A dextrin solution of 0·5 % was used and dextrin was tested for in the external solution by adding 1 drop of N/10 iodine. After 24 hours, no trace appears in 85 %; a trace in 87 %, and this becomes stronger in each successive member of the series. The gradation becomes more rapid towards the upper end of the series.

(*d*) Over the range 92–96 %, the variation of permeability may be shown by use of neutral litmus.

After 10 days' dialysis, the following table gives the strength of dye in the external solutions:

92 %	93 %	94 %	95 %	96 %
0	trace	0·08	0·13	0·33

the internal solutions being of strength 1.

IV. The space relationships of the membrane (amount of water, swelling in all three dimensions) vary continuously with strength of alcohol used.

Gradation is shown over the whole range by measurement of the *water-content*. In making these determinations, the closed end of the membranes was cut away, and the cylindrical portion left was cut open. In determining the wet weights, both surfaces of the membrane were dried rapidly between filter paper; weighing was carried out in a closed bottle.

The gradation is shown in the following table. In each case the weight is given in terms of an original air-dry weight of 100. (Air-dried membranes contain about 3 % water.)

Membrane %	Wet weight after washing	Weight on again drying
0	106·0	100·0
50	111·0	99·3
70	115·6	98·6
80	122·4	98·2
90	141·9	97·0
92	160·5	93·8
94	208·5	89·1
96	387·8	74·8

A parallel series of measurements of the strongly shrinking (5, 1) membranes gave essentially similar results, except that the water percentage in the case of these tended to be higher near the upper end of the series than that of the preceding type.

It will be noticed that the increase in water-content is gradual at first and becomes extremely rapid above 94 %; also that the membrane loses in dry weight with the treatment; that this loss is slight up to 90 %, but becomes very considerable at 96 %.

In the same way the length, circumference and thickness of the air-dried membranes increase continuously when the latter are immersed in alcohol solutions of increasing strength. The following tables show these effects.

(α) *Membranes which show* 10 % *shrinkage on drying.*

The measurements of L (length) and T (thickness) are given on the basis of a length and thickness in the air-dried condition of 100. Longitudinal and circumferential extension have never been found to differ appreciably.

Membrane %	In alcohol before washing		After washing		After drying	
	L	T[1]	L	T	L	T
0	101·7	—	101·7	100·0	100·0	100·0
50	103·7	—	102·0	108·3	97·8	102·8
70	105·8	—	102·0	110·5	—	105·2
80	108·6	—	103·3	118·9	96·7	105·4
90	112·9	—	107·0	162·2	94·7	108·1
92	115·8	—	107·7	200·0	92·1	114·3
94	119·7	—	111·7	280·0	89·5	116·7
96	132·1	—	120·9	362·0	76·7	*

[1] Readings not taken on account of softness of membranes, especially in the higher percentages of alcohol.

* Becomes very crinkled; micrometer reading valueless.

The features brought out by Tables (α) and (β) apart from the continuous gradation are:

(1) Swelling is anisotropic. Thus in 94 % alcohol, the longitudinal extension is 12 %; the extension in thickness is 180 %. This applies to both sets of membranes.

(β) *Membranes which show 30 % shrinkage on drying.*

Membrane %	In alcohol before washing		After washing		After drying	
	L	*T*[1]	*L*	*T*	*L*	*T*
0	102·0	—	101·0	103·5	100·0	100·0
50	104·4	—	[100·5]	107·0	[96·8]	*
70	107·4	—	102·3	124·5	96·7	*
80	108·3	—	102·5	129·6	95·5	*
90	119·7	—	110·0	150·0	94·1	111·0
92	126·8	—	118·3	186·0	—	112·0
94	138·4	—	125·6	300·0	87·2	117·5
96	157·3	—	139·1	410·0	65·1	*

[1] Readings not taken on account of softness of membranes, especially in the higher percentages of alcohol.

* Impossible to obtain a reliable measurement on account of crinkling on drying.

(2) A normal (10 % shrinking) membrane is restored to its original length and circumference by immersion in alcohol of about 94 %. The thickness and, from table on p. 607, the water-content are much increased. Correlated with this is increased permeability of a 94 % membrane as compared with that of the membrane as originally prepared.

(3) With membranes of both types, longitudinal extension is negligible in membranes of 80 % and under; in these the effect of the alcohol treatment shows itself mainly in increase in thickness.

(4) With the higher percentages of alcohol, the strongly shrinking membranes show a higher extension than do the corresponding members of the normal series. Nevertheless the original dimensions are only reached at a higher alcohol percentage, viz. 94–96 %.

(5) The higher the percentage of alcohol employed, the greater is the subsequent shrinkage in length on drying (as shown by comparison of 2nd and 3rd *L* columns in both tables). This increased longitudinal shrinkage is compensated in part by increase in thickness on drying.

While the size relationships above described afford a very complete proof of the continuity in the series of membranes produced by alcohol treatment, their importance must not be over-emphasised. Collodion is a plastic substance, and therefore expansion takes place in the direction which is easiest. The anisotropy above observed is to be explained in this way. It does not at all mean that any kind of structure is to be postulated with regard to the membrane. The higher coefficient of longitudinal expansion of thick than of thin membranes is also to be explained in the same way—the thicker the membrane the more does it tend to expand isotropically, and similarly the thinner the membrane the greater is the tendency for expansion to be

anisotropic. As an example of the latter may be cited some experiments with very thin membranes prepared in a way to be described later in which a longitudinal extension of 3–4 % was accompanied by an increase in thickness of 200 %.

In accordance with this principle, the permeable membranes obtained after alcohol treatment are more uniform in thickness than the dried membranes from which they were derived, and the tendency to uniformity is greater the higher the alcohol percentage.

Uniformity tests.

The diffusion experiments with potassium permanganate tabulated on p. 605 indicate the type of agreement obtaining between the corresponding members of two similarly treated series. More detailed tests of the same type were carried out within the range 70–80 % with methylene blue.

In one experiment, 15 membranes prepared in succession on the same day (membranes not selected in any way and of ordinary uniformity) were treated, 3 in 70 % alcohol, 3 in 72·5 %, and so on with 2·5 % intervals to 80 %. These were set up with methylene blue solution, and the rate of diffusion estimated from day to day by comparing the external liquids with a dilution series of the original methylene blue solution. The members of this series were of strength—1, 2, 4, 8, 16, 32, 64, 128, 256, the last being of strength equal to one-fourth that of the original methylene blue solution. The figures in the following table give the members of this series to which the various dialysates corresponded.

Membrane	1 day	2 days	3 days	4 days	5 days	6 days	7 days
70 (1)	0	0	0	0	0?	2—1	2
70 (2)	0	0	0	0	0	0?	trace
70 (3)	0	0	0	0	0	0?	trace
72·5 (1)	0	2—1	8—4	8	16	32—16	32—
72·5 (2)	0	2—1	4	8—4	8+	16—	16
72·5 (3)	0	2—1	4	8—4	8+	16—	16
75 (1)	trace	16—	32—	32+	64—	64	64+
75 (2)	trace	8	16+	32	64—32	64—	64
75 (3)	trace	8—	16	32—	64—32	64—32	64—
77·5 (1)	8—	32	64—	64	128—64	128—	128—
77·5 (2)	8—4	32	64—	64—	64+	128—64	128—
77·5 (3)	8	64—32	64	64+	128—	128—	128—
80 (1)	16+	64—	64+	64+	128—	128—	128—
80 (2)	16	64—	64+	64+	128—	128—	128—
80 (3)	32—16	64+	128—64	128	128	128	128

After 4–5 days no further dialysis takes place in the 80 % membranes, so that the less permeable 77·5 % membranes overtake them. On examining the liquid inside the 80 % dialysers on the 5th day, it was found that its

strength was in each case about one-eighth that of the original solution. This loss in strength is due to the strong adsorption of dye by the membrane. The adsorptive capacity of these membranes increases with permeability. The amount of dye therefore which passes through the more permeable membranes in the above table is less than would be the case if the internal solutions were kept up to full strength.

A second test on a similarly large scale was carried out, 6 membranes of 75 %, 6 of 77·5 %, and 6 of 80 % being used. The results obtained were essentially the same as in the above case.

Throughout these experiments special attention was directed to the comparison of the most permeable membrane of a given alcohol number with the least permeable of the next higher number. On the basis of these observations, it can be stated that the most permeable membrane of a certain number was in all cases less permeable than the least permeable of those of number 2·5 % higher. Thus whatever be the variations among the individual membranes, these are not sufficient to mask the effects of the differential alcohol treatment—in other words in the region 70–80 % an alcohol treatment which varies by 2·5 % steps definitely produces continuous gradation in an average set of membranes. At the same time however the maximum variation among similarly treated members may be greater than the least gap which obtains between members of two lots the treatment of which differs by 2·5 %. Thus it is probable that with unselected membranes, 2·5 % more or less represents in the region 70–80 % the smallest variation of alcohol treatment which can be reckoned on to mask individual variations and to produce in all cases continuous gradation.

It is reasonable to ascribe the variations here recorded to variations in the original air-dried membranes (especially individual variations in thickness), and therefore it may be expected that with improvement in the method of manufacture a finer gradation will be rendered possible.

At higher points in the series a finer gradation in terms of alcohol percentage is possible. Thus from about 85 % upwards, continuous grading can be obtained by a series of alcohols varying by 1 %. In the region 90–96 %, especially towards the upper end, smaller differences in alcohol treatment will suffice to produce continuous grading. In the region 96–98 % imbibition increases so rapidly with alcohol percentage that a large range of membranes is included within this narrow limit. The accuracy of the grading in this region therefore would probably be found to be limited by the accuracy obtainable in preparing the alcohol mixtures.

From 40–70 % a variation of 10 % in alcohol treatment produces continuous gradation. The air-dried membranes show differences in permeability to simple electrolytes, e.g. NaCl, some practically keeping back this salt, others allowing a very slow diffusion. These initial variations would be expected to influence more markedly the members of lower than of higher alcohol number. The accuracy attainable has not however been sufficiently tested in the case of these membranes of lower permeability.

By the use of this alcohol method, it is possible to prepare a series of membranes the members of which allow of slower and slower diffusion of a given substance. By continuing the series downwards a stage is reached where no diffusion at all of the particular substance takes place. Thus a given substance can be characterised by a number which represents the strength of alcohol required to produce a membrane which just prevents diffusion of the substance. Such a number may be termed the "alcohol index" for the particular substance. Some of these indices have been worked out, and are given in the following table:

Water	} —	Safranine	75–77·5
NaCl, NH_4Cl		Dextrin	85–87·5
$KMnO_4$	30–40	Starch	90
Picric acid	35–40	Aniline blue	92
Potassium oxalate ...	60–70	Litmus (neut.)	93
Bismarck brown ...	65 (—)	Congo red	96
Methylene blue ...	70 (—)	Night blue	>96
Neutral red	72·5–75		

Water, sodium chloride and ammonium chloride all pass through air-dried membranes. Passage of water is shown by endosmosis, also by the fact that water evaporates from a sealed air-dried membrane when the latter is hung up in the air. The rate of passage is small, and individual variations are large in proportion. Some of these membranes only allow the faintest trace of sodium chloride to pass through in a week; in the case of others passage can be detected in two days. It is doubtful if any of these membranes can be prepared so as to allow passage to water, and at the same time to hold back completely simple chlorides.

The strong endosmotic effect is very troublesome in working with such substances as potassium oxalate, especially in molar concentration. The internal liquid tends to overflow and the levels have to be continually readjusted. Most of the crystalloids tested seem to possess a diffusibility similar to that of potassium oxalate. Such were $CuSO_4$, Na_2SO_4, $K_4Fe(CN)_6$. A 60 % membrane allowed a slow diffusion of glucose and of barium chloride. This part of the investigation was not however carried out at all thoroughly.

It should be noted that membranes of index 50–60 % form very efficient semi-permeable membranes, i.e. while easily permeable to water, they hold back a large number of crystalloids. A membrane of this sort may be used to effect a dialytic separation of certain kinds of salts—thus sodium chloride can be readily dialysed away from a sodium chloride, sodium sulphate mixture. Whether such membranes would keep back these crystalloids under pressure remains to be seen.

It might be suggested that the relatively high impermeability of these membranes—say to molar sodium sulphate—is due to the effect of the strong salt solution upon the membrane, viz. to the withdrawal of water from the membrane by the osmotic action of the salt. This however is contradicted by the behaviour of strong sodium chloride solutions which should on this hypothesis have a similar dehydrating effect, by the magnitude of the endosmotic effect, and by the fact that a 60 % membrane after treatment with molar Na_2SO_4 is found to be still of number 60 % in its behaviour to potassium permanganate.

Only a very slow passage of starch is allowed by a 90 % membrane. Even a 92 % membrane to all practical purposes keeps back a starch solution during several days' dialysis. It is probable that many colloids are heterogeneous as regards degree of dispersion, so that a sharply defined "critical point" would not be expected.

The dialysates from 94 and 95 % membranes containing litmus show a peculiar feature. On comparing these with matching dilutions of the internal solution, they are found to give the same colour reaction to acids, but not to alkalis. Thus the addition of a drop of alkali to the matching solution produces a distinct blue; on similar treatment, the dialysates from 94 and 95 % membranes merely become slightly more violet. A similar behaviour, though not so marked, is seen in the dialysate from a 96 % membrane. It is suggested that litmus contains at least two fractions, one of which is more diffusible and gives a part only of the indicator reactions of the crude solution.

Equilibrium.

The question as to whether equilibrium between the collodion membrane and the alcohol solution has been reached in the 24 hours allowed for imbibition, has only been studied in the case of 72·5 % membranes. It was then found that no essential difference in permeability subsisted between membranes which had remained in contact with the alcohol solution for one day and membranes which had been similarly treated for 4 days. Thus in the case

of 72·5 % membranes, a state of equilibrium or zone of slow change has been reached in 24 hours. Higher in the series it is probable that an imbibition equilibrium is reached in a shorter time, but here the slow change due to solution of the membrane becomes more marked. In the case of membranes lower than 70 %, it should not be taken for granted that equilibrium in imbibition is reached after 24 hours' immersion in the alcohol solution.

Uses of these membranes.

Some of the more obvious uses to which these membranes may be put are

(1) replacement of the copper ferrocyanide membranes in osmometric work;

(2) dialysis of intermediate colloids, e.g. dextrin: more generally, the examination and separation of digestion products;

(3) carrying out a more exact study than has yet been made of the relations between the phenomena of diffusion and adsorption.

An experiment of the type suggested in (2) will serve to illustrate the potentialities of the method.

Six 92 % membranes were made and set up as follows:

(1) and (2) 0·1 % starch solution + equal bulk of water,
(3) and (4) 0·5 % dextrin solution + equal bulk of water,
(5) and (6) 0·5 % dextrin solution + 0·1 % starch solution in equal bulks.

These were tested each day over a period of four days, when it was found that in no case could starch be detected in the dialysates, whereas dextrin of strength 0·05 % dialysed through in each 24 hours in the case of membranes (3), (4), (5) and (6).

On the fifth day, each dialyser was washed and set up alongside a 50 % and an 85 % membrane, with a mixture in equal parts of the following:

0·5 % starch solution,
0·5 % dextrin solution,
0·2 m glucose solution,
1 m sodium chloride solution.

After 24 hours' dialysis, the following scheme represents the condition of the dialysates:

Test	50 %	85 %	92 %	Internal
$AgNO_3$	fairly strong precipitate	strong precipitate	strong precipitate	strong precipitate
Fehling's solution	neg.	strong reduction	—	—
I_2 in KI	coloration = water control	coloration = water control	strong red coloration	intense blue

The application of these membranes to more difficult problems of analysis may be anticipated.

All the work hitherto described was carried out with an 8 % collodion solution in equal parts of ether and alcohol. Other collodion solutions have been tested so far as to indicate what possibilities they present.

Acetone collodion; 8 % solution; dispersion takes place with rapidity, giving a solution which is more mobile than the corresponding ether-alcohol solution.

The membranes prepared from this solution in the ordinary way are white throughout, show normal amount of irregularity in thickness in different parts, have a small water percentage and comparatively low permeability in wet condition and show small shrinkage (6 %) on drying. In the wet condition the thickness is 8–10 μ, and after drying 5–7 μ.

When treated by the alcohol method, a more gradual variation with alcohol percentage is obtained, the interval 0 %—70 % in the case of ether-alcohol membranes corresponding with the interval 0 %—90 % with acetone membranes.

These membranes appear to be of practical utility, especially in respect of their more gradual grading below 90 %.

Ether collodion; 8 % solution; dispersion does not occur so rapidly as in acetone; the resultant solution is very viscous, more so than the ether-alcohol solution. The membranes were prepared in the usual way; they are white, give extremely small shrinkage on drying, and when freshly prepared are permeable only to water and a few salts. They are extremely difficult to prepare on account of the high impermeability which prevents the rapid access of water to the underlying layers. By the action of water the collodion film becomes hard and can with difficulty be removed from the tube.

On treatment with alcohol, these membranes show a behaviour similar to that of the acetone-collodion membranes described above, i.e. they respond more sluggishly to the alcohol treatment.

This last feature is considered to be of interest in respect of the general technique adopted. It would explain the tendency observed in certain membranes to be more permeable towards the closed end, where, on account of the conditions obtaining during their manufacture, they tend to contain a lower percentage of ether at the time of fixation in water.

Alcohol collodion. The 8 % solution was so viscous that a 4 % solution was employed. The latter is rather more fluid than the normal 8 % ether-alcohol solution.

The membranes were held for a few seconds in air, and then fixed in

water. They are glass-clear and in the wet condition are highly permeable, e.g. to starch. In spite of the thin solution employed, wet membranes are extremely thick (200–300 μ), and have a very high water-content (dry weight = 100, wet weight = 1400). The shrinkage on drying is enormous (60–70 %).

The interest of these membranes is that they form the end term of the series; ether membrane → membrane of type (5, 0) → membrane of type (5, 1) → alcohol membrane.

Gradation by means of acetone-water mixtures.

No practicable results can be got by using acetone-water in place of alcohol-water mixtures. In 60 % acetone (and higher concentrations) membranes of any type become unmanageably soft (the clear normal type of membrane becoming white in this process); in 40 % acetone, the membranes soften, shrink to very small dimensions, and on transferring to water become hard and highly impermeable.

Membranes made inside the tube.

These can be prepared conveniently as follows. Two tubes of the same diameter are passed through a cork as in Fig. 1. One of these is filled with 8 % collodion solution. On turning upside down and allowing to drain, a film is left inside the upper tube. Fifteen minutes drainage was allowed. The tube with the film is then taken out, and air blown in by means of a pipette until the whole film is nearly dry (one minute is about sufficient); the free end of the membrane is now disengaged with a knife and a little further blowing causes the whole membrane to come away. These membranes are simply dried out in air without coming in contact with water. They are of the clear alcohol type, but do not show shrinkage as they had already almost dried while in contact with the glass, which restrains shrinkage in a mechanical way.

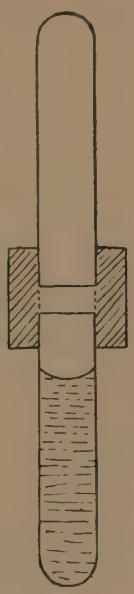

Fig. 1

These membranes can with skill be prepared of great uniformity (apart from the usual tendency to be thinner at the closed than at the open end), they are very thin, they can be differentiated in alcohol in the usual way, and their use is recommended in cases where a rapid dialysis is imperative and where adsorption of a particular substance on the membrane is required to be

reduced as much as possible. These membranes, on account of their extreme thinness, are rather fragile.

Thin sheet collodion can be readily prepared in this way, viz. by forming a membrane inside a large cylindrical vessel and treating in the above manner. After drying, the film is simply cut down one side with a knife and taken out.

Method of grading membranes by controlling the amount of drying.

This method was the one first adopted. It bears certain resemblances to Schoep's method. In fact, in the early stages, glycerol solutions were used, the wet membranes as prepared in the first instance being soaked in glycerol solutions of different strengths and then hung up in air to dry. The higher the percentage of glycerol the greater the permeability of the resultant membrane.

This method was later superseded by the following. A solution of $CaCl_2$, as nearly as possible saturated and with alkalinity neutralised, was prepared, filtered, and placed in the bottom of a large desiccator. This solution may be designated *A*. The wet membranes were soaked for 24 hours in various dilutions of *A*, and then hung up over the concentrated solution in the desiccator; the latter is then exhausted. By this means the attainment of equilibrium is expedited. It is obvious that a membrane which has imbibed a 5 % dilution of *A* loses more water in reaching equilibrium with *A* than a membrane which has been soaked in a 10 % dilution, and would accordingly be expected to be less permeable. The membranes are finally washed and are then ready for use.

The practical features of the process are as follows:

The $CaCl_2$ solution should be as strong as possible: with weak solutions the attainment of equilibrium is slower. Before the membranes are hung up to dry in the desiccator, all excess drops of the diluted $CaCl_2$ solution must be wiped off. Only in this way can uniformity be obtained.

As regards results, this method is distinctly good. It is necessary however that the membranes before treatment be of uniform permeability throughout. Any marked variations will be reproduced in the graded membranes. In fact in the present work the variations in different parts of the freshly prepared membranes as described on p. 596 were first discovered by the variations which appeared in different parts of the membranes, the permeability of which had been reduced in this way. In order to secure uniform membranes to begin with, the freshly prepared membranes were soaked in a 94 % alcohol solution.

A membrane which has been soaked in 94 % alcohol, then graded down by using a 7·5 % *A* solution just holds back methylene blue, i.e. it corresponds to a membrane treated with 70 % alcohol.

Apart from its laboriousness and its limited applicability, this method may be objected to inasmuch as the removal of the calcium chloride from the graded membrane may be slow, and may not take place at all in some cases. This objection could be met by using strong solutions of sodium chloride instead. There is a possibility that such a method may be of use in preparing membranes for osmometric work.

Summary.

Collodion thimbles of regularly increasing degree of permeability may be prepared by soaking air-dried thimbles in alcohol-water mixtures of increasing alcohol content.

The diffusive capacity of any substance through collodion may be specified in terms of the alcohol strength required to produce the membrane which just prevents its passage. This may be termed the "alcohol index" of the substance.

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